

AD _____

Award Number: DAMD17-03-2-0031

TITLE: Evaluation of Chemicals for Antimalarial Activity Against Blood and Tissue Stages

PRINCIPAL INVESTIGATOR: Arba Ager, Ph.D.

CONTRACTING ORGANIZATION: University of Miami
Miami, FL 33177

REPORT DATE: July 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 01-07-2005			2. REPORT TYPE Annual			3. DATES COVERED (From - To) 14 Mar 2004 - 31 May 2015		
4. TITLE AND SUBTITLE Evaluation of Chemicals for Antimalarial Activity Against Blood and Tissue Stages						5a. CONTRACT NUMBER		
						5b. GRANT NUMBER DAMD17-03-2-0031		
						5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Arba Ager, Ph.D.						5d. PROJECT NUMBER		
						5e. TASK NUMBER		
						5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Miami Miami, FL 33177						8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012						10. SPONSOR/MONITOR'S ACRONYM(S)		
						11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited								
13. SUPPLEMENTARY NOTES								
14. ABSTRACT Three different test systems were used to detect antimalarial activity of new compounds in mice. The first test (AT Test) detected activity against blood stage parasites (Thompson Test). There were 105 three level tests done plus 3 one level tests performed. 54 compounds exhibited activity against the asexual blood stages. The 2 most active compounds were BQ 99377 and BR 01050. The second test (SM Test) detected activity against the sporozoite stages or the exoerythrocytic schizont stages in the liver. There were 66 three level tests done where the drug was given once and 2 tests where the drugs were given for 3 days. The most active compounds were BQ 11373, BQ 93713, BQ 93722, BH 50615, BH 58522 AND BR 01069. There were 18 SM tests done to detect synergistic activity between Primaquine and methylene blue, Atovaquone, Proguanil, Dapsone, Quinine, BQ 98852 and Doxycycline. The third test detects activity against the gametocyte stages. There were 12 compounds tested. The new oracle database was used to tabulate and analyze data from both the AT and SM test systems.								
15. SUBJECT TERMS Malaria, chemotherapy, Plasmodium, blood & tissue schizonticides, Gametocytes								
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 25	19a. NAME OF RESPONSIBLE PERSON			
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)			

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	6
Appendices.....	6

Malaria continues to be a major killer in many parts of the world today with over 500 million people becoming infected with malaria resulting in over 3 million deaths annually. Prevention of malaria is hindered by several factors including drug-resistant parasites and insecticide-resistant mosquitoes. Military troops are stationed in many different malaria infested areas of the world today and there are very few effective drugs that can be used. These drugs have problems with drug resistance and toxicity. Therefore, new antimalarial agents are constantly needed to stay one step ahead in the control and prevention of this elusive malarial parasite.

In an effort to identify new antimalarial compounds active against different stages of the malarial life cycle 3 different test systems were used. The first is the Army Thompson Test System (AT Test System) identifies drugs active against the asexual blood stages of *Plasmodium berghei*. The second is the Sporozoite Miami Test System (SM Test System) identifies drugs with activity against the sporozoite and/or the exoerythrocytic liver tissue stages of *Plasmodium yoelii*. The third is the Antigametocyte Test System (GT) identifies drugs that cause gametocytes to be removed from the blood of mice infected with *P. yoelii*. In the AT Test System compounds were given to mice bid for 3 days after the mice were infected with blood stage parasites to determine activity against developing asexual blood parasites. In the SM Test System test compounds are administered to mice once 4 hr before they received sporozoites of *P. yoelii* (obtained from *Anopheles stephensi* mosquitoes) in order to determine activity against the sporozoite stage in the blood of mice or the subsequent developing exoerythrocytic schizont tissue stages in the liver. A compound identified active in this test system could be utilized as a prophylactic drug to prevent a person from ever getting infected. In the AG Test System compounds are given to mice once on the 5th day after infection for with *P. yoelii* and their blood is examined for the presence of gametocytes 1 day later.

BODY

One hundred and five compounds were tested at 3 dose levels and 3 compounds at 1 level for activity against asexual blood stage parasites in mice infected with *P. berghei* (AT Test System). Activity was measured by decreases in parasitemias, extension of survival times and cures. There were 54 compounds that were found to have antimalarial activity in this system. These compounds are tabulated in Table I. We were to evaluate 150 compounds during this contract period in this test system but did not because we were sent only 105. During the second year we plan on testing 150 compounds.

Sixty-eight 3-level tests were done to detect activity against sporozoite stages or tissue exoerythrocytic stages in the liver of mice inoculated with sporozoites of *P. yoelii* (SM Test System). Activity was determined by decreased parasitemias. Forty-five compounds had activity. Three very active compounds were BQ 11373, BQ 29286 and BQ 35131. In the SM synergy tests we found possible synergy between Primaquine and Dapsone and Proguanil. The other tests were additive in effect. These 18 tests amounted

to 90 three level tests so we performed 158 three level tests. We were to do 150 three level tests during this contract period in this test system but did 158. During the third year of this contract we plan to perform 150 three level tests. The new data entry system from Oracle worked fine for the regular 3 level SM tests but needs to be modified to analyze the SM synergy tests.

One hundred and twenty nine compounds tested at 1 or more levels in the gametocyte test (GT). The results of these tests are currently being analyzed.

We did not test any compounds in any of the drug-resistant lines of malaria because we were not requested to do so.

KEY RESEARCH ACCOMPLISHMENTS

- 1) Performed 105 three-level tests for antimalarial activity against blood stages of *P. berghei* in mice (AT Test System). There were 54 active compounds. All compounds tested in this system are summarized in Table I. The 2 most active compounds are BQ 99377 and BR 01050.
- 2) Performed 158 three-level tests for antimalarial activity against the sporozoite stage or tissue stages in the liver of mice infected with sporozoites of *P. yoelii* obtained from *A. stephensi* mosquitoes (SM Test). The most active compounds were BQ 11373, BQ 93713, BQ 93722, BH 50615, BH 58522 AND BR 01069.
- 3) Developed a new test to detect activity against the gametocyte stages (GT Test System) and tested 129 compounds for activity.
- 4) Tested a new Oracle data base developed by WRAIR for data obtained in the AT and SM Test Systems until it worked for the following activities measured; mortality, mouse body weights, survival times, and parasitemia values.

REPORTABLE OUTCOMES

A new Oracle database developed by WRAIR for entering and analyzing data obtained from the AT and SM Test Systems was tested and modified until it worked satisfactorily. This database allows one enter data as it is obtained directly into the database and analyzes it. Such data includes mouse body weights, drugs and their dosages, clinical observations of the mice, parasitemias and mortality values. A similar database is being developed for the data obtained from the drug combination tests in the SM Test System.

CONCLUSIONS

In the Army Thompson Test System (AT) 105 three-level tests were performed with 54 compounds identified as active in attacking the asexual blood stage parasites that are the stages which cause the pathology during a malarial infection.

In the Sporozoite induced SM Test System 158 three-level tests were performed and identified 45 compounds as active in attacking either the sporozoite stages or the developing exoerythrocytic schizont stages that would prevent a malarial infection in a person.

A new test to detect activity against the gametocyte stages of malaria (GT) was performed on 129 compounds with the hope of finding one to block the transmission of malaria to the mosquito.

The Oracle Data Base System has been used to enter and analyze data for the both the AT and SM Test Systems. This data base will allow all of the data in this system to be computerized and made available to Army researchers at Walter Reed on a regular basis.

REFERENCES

There were no publications during this contract period.

APPENDICES

Table 1 Results of Compounds Tested in the Army Thompson Test System (AT)

TEST	COMPOUND	DOSE LEVELS	# DAYS	ROUTE	VEHICLE	DOSING
						#
38	BQ 92387	160, 40, 10	3	ORAL	NACL	B D
	BQ 92396	160, 40, 10	3	ORAL	NACL	B D
	BQ 92387	160, 40, 10	3	SC	NACL	B D
	BQ 92396	160, 40, 10	3	SC	NACL	B D
	BQ 37617	10, 5, 2.5	3	ORAL	HEC	B D
39	AY 71053	160, 40, 10, 2.5	3	ORAL	HEC	B D
	AJ 78301	40, 20, 10, 5, 2.5, 1.25	3	ORAL	HEC	ONCE A DAY
	BE 21913	40, 20, 10, 5, 2.5, 1.25	3	ORAL	HEC	ONCE A DAY
	BQ 92261	640, 160, 40	5	ORAL	HEC	ONCE A DAY
	BQ 92270	640, 160, 40	5	ORAL	HEC	ONCE A DAY
	BQ 92289	640, 160, 40	5	ORAL	HEC	ONCE A DAY
	BQ 92298	640, 160, 40	5	ORAL	HEC	ONCE A DAY
	BQ 92305	640, 160, 40	5	ORAL	HEC	ONCE A DAY
40	BQ 95655	160, 40, 10	3	ORAL	HEC	B D
	BQ 95664	160, 40, 10	3	ORAL	HEC	B D
	AY 71053	2.5, 1.25, 0.625, 0.3125	3	ORAL	HEC	ONCE A B D
	BE 07379	1.25, 0.625, 0.3125	3	ORAL	HEC	ONCE A DAY
	AH 73945	1.25, 0.625, 0.3125	3	ORAL	HEC	ONCE A DAY
41	ZA 04312	1.25, 0.625, 0.3125	5	ORAL	HEC	B D
	BE 21824	1.25, 0.625, 0.3125, 0.156	3	ORAL	HEC	B D
42	AU 29291	128, 64, 32, 16, 8, 4, 2	3	ORAL	HEC	B D
	BK 11592	128, 32, 8, 2	3	ORAL	HEC	B D
	BJ 23346	128, 32, 8, 2	3	ORAL	HEC	B D
43	BK 11592	2, 1, 0.5, 0.25	3	ORAL	HEC	B D
	BJ 23346	2, 1, 0.5, 0.25, 0.125, 0.0625	3	ORAL	HEC	B D
	AY 71053	2.5, 1.25, 0.625, 0.3125, 0.156	3	ORAL	HEC	B D
44	BQ 96027	40, 20, 10	3	ORAL	HEC	B D

	BQ 96036	40, 20, 10	3	ORAL	HEC	B D
	BQ 96045	40, 20, 10	3	ORAL	HEC	B D
	BQ 96054	640, 20, 10, 5	3	ORAL	HEC	B D
	AU 29291	16, 4	3	ORAL	HEC	B D
555	BQ 97275	512, 128, 32, 8	3	ORAL	HEC	B D
557	BM 18411	80, 20, 5	3	ORAL	HEC	B D
	BQ 33477	80, 20, 5	3	ORAL	HEC	B D
	BQ 97944	80, 20, 5	3	ORAL	HEC	B D
	BQ 97953	80, 20, 5	3	ORAL	HEC	B D
	BQ 97962	80, 20, 5	3	ORAL	HEC	B D
559	BM 01916	512, 256, 128	3	ORAL	HEC	B D
	BQ 35417	256, 128, 64	3	ORAL	HEC	B D
	BQ 93517	256, 128, 64	3	ORAL	HEC	B D
	BQ 95860	256, 128, 64	3	ORAL	HEC	B D
	BQ 97944	320	3	ORAL	HEC	B D
	BQ 97953	320	3	ORAL	HEC	B D
	BQ 97962	320	3	ORAL	HEC	B D
561	BQ 33477	5, 2.5, 1.25	3	ORAL	HEC	B D
	BM 01916	128, 64, 32	3	ORAL	HEC	B D
	BQ 98094	160, 40, 10	3	ORAL	HEC	B D
	BQ 35533	256, 128, 64	3	ORAL	HEC	B D
	ZW 61193	256, 128, 64	3	ORAL	HEC	B D
566	BQ 98094	160, 40, 10	3	ORAL	HEC	B D
	BQ 96045	320, 160, 80	3	ORAL	HEC	B D
	BQ 95048	320, 80, 20	3	SC	P. OIL	B D
	BQ 95048	320, 80, 20	3	ORAL	HEC	B D
	BQ 99377	80, 20, 5	3	ORAL	HEC	B D
570	BQ 96045	40, 20, 10	3	ORAL	HEC	B D
	BQ 99377	5, 2.5, 1.25	3	ORAL	HEC	B D
	BR 01050	80, 20, 5, 2.5, 1.25	3	ORAL	HEC	B D
	BR 01096	80, 20, 5, 2.5	3	ORAL	HEC	B D
575	BQ 99377	1.25, 0.625, 0.3125, 0.156	3	ORAL	HEC	B D
	BR 01050	1.25, 0.625, 0.3125, 0.156	3	ORAL	HEC	B D
	BR 01096	2.5, 1.25, 0.625, 0.3125, 0.156	3	ORAL	HEC	B D
	AU 29291	64, 4	3	ORAL	HEC	B D
593	BQ 91317	320, 80, 20	3	IP	NACL	B D
577	BQ 91317	320, 80, 20	3	SC	P.OIL	B D
	BM 18644	320, 80, 20, 10	3	SC	P.OIL	B D
593	BM 18644	320, 80, 20, 10	3	IP	NACL	B D

582	BR 50482	160, 40, 10	3	SC	P.OIL	B D
595	BR 01050	0.156, 0.078, 0.039	3	ORAL	HEC	B D
584	BR 51407	320, 80, 20	3	SC	P.OIL	B D
	BR 51416	320, 80, 20	3	SC	P.OIL	B D
	BR 51425	320, 80, 20	3	SC	P.OIL	B D
	BR 51434	320, 80, 20	3	SC	P.OIL	B D
	BR 51443	320, 80, 20	3	SC	P.OIL	B D
	BR 51452	160, 80, 20	3	SC	P.OIL	B D
	BR 51461	320, 80, 20	3	SC	P.OIL	B D
	BR 51470	320, 80, 20	3	SC	P.OIL	B D
	BR 51489	320, 80, 20	3	SC	P.OIL	B D
	BR 51498	320, 80, 20	3	SC	P.OIL	B D
	BR 51505	320, 80, 20	3	SC	P.OIL	B D
	BR 51514	320, 80, 20	3	SC	P.OIL	B D
	BR 36919	320, 80, 20	3	SC	P.OIL	B D
586	BR 51407	320, 80, 20	3	ORAL	HEC	E D
	BR 51416	320, 80, 20	3	ORAL	HEC	E D
	BR 51425	160, 40, 10	3	ORAL	HEC	E D
	BR 51434	320, 80, 20	3	ORAL	HEC	E D
	BR 51443	320, 80, 20	3	ORAL	HEC	E D
	BR 51452	160, 80, 20	3	ORAL	HEC	E D
	BR 51461	320, 80, 20	3	ORAL	HEC	E D
	BR 51470	320, 80, 20	3	ORAL	HEC	E D
	BR 51489	320, 80, 20	3	ORAL	HEC	E D
	BR 51498	320, 80, 20	3	ORAL	HEC	E D
	BR 51505	320, 80, 20	3	ORAL	HEC	E D
	BR 51514	320, 80, 20	3	ORAL	HEC	E D
	BR 36919	320, 80, 20	3	ORAL	HEC	E D
590	BR 51532	640, 160, 40	3	SC	P.OIL	E ID
	BR 51541	640, 160, 40	3	SC	P.OIL	E ID
	AB 53130	160, 40, 10	3	SC	P.OIL	E ID
593	BQ91317	320, 80, 20	3	IP	SALINE	E ID
	BM18644	320, 80, 20	3	IP	SALINE	E ID
604	BR 01050	0.78, 0.39	3	ORAL	HEC	E ID
	BR 51550	640, 160, 40	3	ORAL	HEC	E ID

Table II Results of Compounds Tested in the Regular SM Test

TEST	COMPOUND	DOSE LEVELS	ROUTE	VEHICL E	ACTIVE YES OR NO
#	#	MG/KG			
31	BQ 11373	10, 5, 2.5	ORAL	HEC	YES
	BQ 91611	160, 40, 10	ORAL	HEC	NO
	BJ 23346	160, 40, 10	ORAL	HEC	YES
	AU 86545	160, 40, 10	SC	P.OIL	YES
	ZM 87843	160, 40, 10	ORAL	HEC	YES
33	BQ 93713	160, 40, 10	ORAL	HEC	YES
	BQ 93722	160, 40, 10	ORAL	HEC	YES
	BQ 93731	160, 40, 10	ORAL	HEC	YES
	ZB 69096	160, 40, 10	ORAL	HEC	YES
	BJ 08241	160, 40, 10	ORAL	HEC	YES
34	BQ 93713	10, 5, 2.5	ORAL	HEC	YES
	BQ 93722	10, 5, 2.5	ORAL	HEC	YES
	BQ 93731	160, 40, 10	ORAL	HEC	YES
35 =					
569	BQ 93713	2.5, 1.25, 0.625	ORAL	HEC	NO
	AY 71053	160, 40, 10, 2.5	ORAL	HEC	YES
36	BJ 23346	10, 5, 2.5	ORAL	HEC	YES
	AU 86545	10, 5, 2.5	SC	P.OIL	YES
39	BJ 23346	10, 5, 2.5	ORAL	HEC	NO*
40	BJ 23346	160, 40, 10	ORAL	HEC	YES
	AW 23628	160, 40, 10	ORAL	HEC	YES
	SULFANILAMIDE	160, 40, 10	ORAL	HEC	YES
41	BH 47832	160, 40, 10	ORAL	HEC	
	BH 50615	160, 40, 10	ORAL	HEC	
	BH 58522	160, 40, 10	ORAL	HEC	
	AY 65859	160, 40, 10, 2.5, 0.625, 0.156	ORAL	HEC	
42	BH 50615	10, 5, 2.5, 1.25, 0.625, 0.3125	ORAL	HEC	YES
	BH 58522	10, 5, 2.5, 1.25, 0.625, 0.3125	ORAL	HEC	YES
	CHLOROQUINE	160, 40, 10	ORAL	HEC	NO
543	BJ 08241	40, 20, 10	ORAL	HEC	
571	BR 01069	80, 40, 20, 10, 5	ORAL	HEC	YES
	BR 01078	80, 40, 20, 10, 5	ORAL	HEC	YES
	BR 01087	80, 40, 20, 10, 5	ORAL	HEC	YES

572	BR 16444 BR 01103 BK 73252	160, 80, 40, 20, 10 80, 40, 20, 10, 5 80, 40, 20, 10, 5	ORAL ORAL ORAL	HEC HEC HEC	NO YES YES
573	BR 01103 BR 01069 BR 01078	20, 10, 5, 2.5 20, 10, 5, 2.5 20, 10, 5, 2.5	ORAL ORAL ORAL	HEC HEC HEC	YES YES YES
574	AU 96336 BN 36843	160, 40, 10 40, 10, 2.5, 0.625	ORAL ORAL	HEC HEC	YES NO
580	BE 50003 AU 96336 BN 36843 AG 64950	160, 40, 10, 2.5 160, 40, 10 40, 10, 2.5, 0.625 160, 40, 10, 2.5	ORAL ORAL ORAL ORAL	HEC HEC HEC HEC	YES NO NO NO
585	BD 57427 BG 66412 BG 56023 ZM 33185 AW 23628	10, 2.5, 0.625 2.5, 1.25, 0.625 2.5, 1.25, 0.625 2.5, 1.25, 0.625 160, 40, 10	ORAL ORAL ORAL ORAL ORAL	HEC HEC HEC HEC HEC	NO NO NO NO YES
587	BH 35770 BH 67852 BG 60741 BJ 91335 BJ 45646	160, 40, 10 160, 40, 10 160, 40, 10 160, 40, 10 160, 40, 10	ORAL ORAL ORAL ORAL ORAL	HEC HEC HEC HEC HEC	YES YES YES YES NO
589	BD 57954 BD 57972 BD 58817	160, 40, 10 160, 40, 10 160, 40, 10	SC SC SC	P.OIL P.OIL P.OIL	NO YES NO
603	BH 50615	160, 40, 10	ORAL	HEC	YES
592	AL 72014 AJ 58050	160, 40, 10 160, 40, 10	ORAL ORAL	HEC HEC	YES YES
594	AQ 29997 BJ 42681 AE 58434	160, 40, 10 160, 40, 10 320, 80, 20	ORAL ORAL ORAL	HEC HEC HEC	YES YES NO

Table III Compounds Tested in SM Combination Tests for Synergism

TEST #	COMPOUND #	MG/KG	DOSING ON DAY(S)	ROUTE	VEHICLE	ACTIVE YES/NO
37	PRIMAQUINE (P) BJ 08241	100 50 25 12.5	0 0 0 0	ORAL ORAL ORAL ORAL	HEC HEC HEC HEC	YES YES NO NO
	METHYLENE BLUE (MB)	50 25 12.5	0 0 0	ORAL ORAL ORAL	HEC HEC HEC	NO NO NO
	P + MB	100 + 50 50 + 50 25 + 50 12.5 + 50	0 0 0 0	ORAL ORAL ORAL ORAL	HEC HEC HEC HEC	YES YES NO NO
	P + MB	100 + 25 50 + 25 25 + 25 12.5 + 25	0 0 0 0	ORAL ORAL ORAL ORAL	HEC HEC HEC HEC	YES YES NO NO
	RESULTS - ADDITIVE EFFECT					
38	PRIMAQUINE (P) BJ 08241	50 25 12.5	0 0 0	ORAL ORAL ORAL	HEC HEC HEC	YES NO NO
	METHYLENE BLUE (MB) AT 54995	100	0	ORAL	HEC	NO
	P + MB	50 + 100 25 + 100 12.5 + 100	0 0 0	ORAL ORAL ORAL	HEC HEC HEC	YES NO NO
	RESULTS - ADDITIVE EFFECT					
543	PRIMAQUINE (P) BJ 08241	40 20 10	0 0 0	ORAL ORAL ORAL	HEC HEC HEC	YES YES YES
	ATOVAQUONE (A)	40 10 2.5	0 0 0	ORAL ORAL ORAL	HEC HEC HEC	YES YES YES

P + A	40 + 40	0	ORAL	HEC	YES
	20 + 10	0	ORAL	HEC	YES
	10 + 2.5	0	ORAL	HEC	YES

RESULTS - NOT DETERMINED NEED LOWER DOSES

544	PRIMAQUINE (P)	40	0	ORAL	HEC	YES
	BJ 08241	20	0	ORAL	HEC	NO
		10	0	ORAL	HEC	NO
		5	0	ORAL	HEC	NO
		2.5	0	ORAL	HEC	NO
	ATOVAQUONE (A)	2.5	0	ORAL	HEC	YES
		1.25	0	ORAL	HEC	YES
		0.625	0	ORAL	HEC	YES
		0.3125	0	ORAL	HEC	YES
		0.156	0	ORAL	HEC	YES
	P + A	40 + 2.5	0	ORAL	HEC	YES
		20 + 1.25	0	ORAL	HEC	YES
		10 + 0.625	0	ORAL	HEC	YES
		5 + 0.3125	0	ORAL	HEC	YES
		2.5 + 0.156	0	ORAL	HEC	YES

RESULTS - NOT DETERMINED NEED LOWER DOSES

545	PRIMAQUINE (P)	40	0	ORAL	HEC	YES
	BJ 08241	20	0	ORAL	HEC	NO
		10	0	ORAL	HEC	NO
		5	0	ORAL	HEC	NO
		2.5	0	ORAL	HEC	NO
	ATOVAQUONE (A)	0.156	0	ORAL	HEC	YES
		0.078	0	ORAL	HEC	YES
		0.039	0	ORAL	HEC	NO
		0.0195	0	ORAL	HEC	NO
		0.00975	0	ORAL	HEC	NO
	P + A	40 + 0.156	0	ORAL	HEC	YES
		20 + 0.078	0	ORAL	HEC	YES
		10 + 0.039	0	ORAL	HEC	NO
		5 + 0.0195	0	ORAL	HEC	NO
		2.5+0.0097	0	ORAL	HEC	NO
		5	0	ORAL	HEC	NO

RESULTS - ADDITIVE EFFECT

45	PRIMAQUINE (P)	20	0	ORAL	HEC	YES
		10	0	ORAL	HEC	NO
	ATOVAQUONE (A)	0.156	0	ORAL	HEC	YES

		0.078	0	ORAL	HEC	YES
		0.039	0	ORAL	HEC	NO
P + A	20 + 0.078	0	ORAL	HEC	YES	
		0	ORAL	HEC	YES	
		0	ORAL	HEC	YES	
		0	ORAL	HEC	YES	
		0	ORAL	HEC	NO	

RESULTS - SYNERGISTIC EFFECT

553	PRIMAQUINE (P)	20	-1, 0, +1	ORAL	HEC	NO
	BJ 08241	5	-1, 0, +1	ORAL	HEC	NO
		1.25	-1, 0, +1	ORAL	HEC	NO
	PROGUANIL	20	-1, 0, +1	ORAL	HEC	YES
	AG 64923	5	-1, 0, +1	ORAL	HEC	YES
		1.25	-1, 0, +1	ORAL	HEC	NO
	DOXYCYCLINE	20	-1, 0, +1	ORAL	HEC	NO
	BL 51822	5	-1, 0, +1	ORAL	HEC	NO
		1.25	-1, 0, +1	ORAL	HEC	NO
	α-ARTESUNIC ACID	20	-1, 0, +1	ORAL	HEC	NO
	BM 17174	5	-1, 0, +1	ORAL	HEC	NO
		1.25	-1, 0, +1	ORAL	HEC	NO
	DAPSONE	20	-1, 0, +1	ORAL	HEC	NO
	ZB 25290	5	-1, 0, +1	ORAL	HEC	NO
		1.25	-1, 0, +1	ORAL	HEC	NO
556	PRIMAQUINE	80	-1, 0, +1	ORAL	HEC	YES
	BJ 08241	40	-1, 0, +1	ORAL	HEC	YES
		20	-1, 0, +1	ORAL	HEC	NO
	DOXYCYCLINE	160	-1, 0, +1	ORAL	HEC	YES
	BL 51822	80	-1, 0, +1	ORAL	HEC	NO
		40	-1, 0, +1	ORAL	HEC	NO
	α-ARTESUNIC ACID	160	-1, 0, +1	ORAL	HEC	NO
	BM 17174	80	-1, 0, +1	ORAL	HEC	NO
		40	-1, 0, +1	ORAL	HEC	NO
	QUININE	160	-1, 0, +1	ORAL	HEC	NO
	BM 05870	80	-1, 0, +1	ORAL	HEC	NO
		40	-1, 0, +1	ORAL	HEC	NO
	DAPSONE	80	-1, 0, +1	ORAL	HEC	YES

	ZB 25290	40	-1, 0, +1	ORAL	HEC	NO
		20	-1, 0, +1	ORAL	HEC	NO
560	PRIMAQUINE (P)	20	-1, 0, +1	ORAL	HEC	NO
	BJ 08241	10	-1, 0, +1	ORAL	HEC	NO
		5	-1, 0, +1	ORAL	HEC	NO
	PROGUANIL (PROG)	2.5	-1, 0, +1	ORAL	HEC	YES
	AG 64923	1.25	-1, 0, +1	ORAL	HEC	YES
		0.625	-1, 0, +1	ORAL	HEC	YES
	P + PROG	20 + 2.5	-1, 0, +1	ORAL	HEC	YES
		20 + 1.25	-1, 0, +1	ORAL	HEC	YES
		20 + 0.625	-1, 0, +1	ORAL	HEC	YES
		10 + 2.5	-1, 0, +1	ORAL	HEC	YES
		10 + 1.25	-1, 0, +1	ORAL	HEC	YES
		10 + 0.625	-1, 0, +1	ORAL	HEC	YES
		5 + 2.5	-1, 0, +1	ORAL	HEC	YES
		5 + 1.25	-1, 0, +1	ORAL	HEC	YES
		5 + 0.625	-1, 0, +1	ORAL	HEC	YES

RESULTS - NOT DETERMINED NEED LOWER LEVELS

562	PRIMAQUINE (P)	20	-1, 0, +1	ORAL	HEC	NO
	BJ 08241	10	-1, 0, +1	ORAL	HEC	NO
		5	-1, 0, +1	ORAL	HEC	NO
	DAPSONE (D)	20	-1, 0, +1	ORAL	HEC	YES
	ZB 25290	10	-1, 0, +1	ORAL	HEC	YES
		5	-1, 0, +1	ORAL	HEC	NO
	P + D	20 + 20	-1, 0, +1	ORAL	HEC	YES
		20 + 10	-1, 0, +1	ORAL	HEC	YES
		20 + 5	-1, 0, +1	ORAL	HEC	YES
		10 + 20	-1, 0, +1	ORAL	HEC	YES
		10 + 10	-1, 0, +1	ORAL	HEC	YES
		10 + 5	-1, 0, +1	ORAL	HEC	YES
		5 + 20	-1, 0, +1	ORAL	HEC	YES
		5 + 10	-1, 0, +1	ORAL	HEC	YES
		5 + 5	-1, 0, +1	ORAL	HEC	YES

RESULTS - SYNERGISTIC EFFECT

564	PRIMAQUINE (P)	20	-1, 0, +1	ORAL	HEC	YES
	BJ 08241	10	-1, 0, +1	ORAL	HEC	YES
		5	-1, 0, +1	ORAL	HEC	NO
	QUININE (Q)	80	-1, 0, +1	ORAL	HEC	NO
	BM 05870	40	-1, 0, +1	ORAL	HEC	NO

	20	-1, 0, +1	ORAL	HEC	NO
P + Q	20 + 80	-1, 0, +1	ORAL	HEC	TOXIC
	20 + 40	-1, 0, +1	ORAL	HEC	TOXIC
	20 + 20	-1, 0, +1	ORAL	HEC	TOXIC
	10 + 80	-1, 0, +1	ORAL	HEC	TOXIC
	10 + 40	-1, 0, +1	ORAL	HEC	YES
	10 + 20	-1, 0, +1	ORAL	HEC	YES
	5 + 80	-1, 0, +1	ORAL	HEC	YES
	5 + 40	-1, 0, +1	ORAL	HEC	YES
	5 + 20	-1, 0, +1	ORAL	HEC	NO

RESULTS - SYNERGISTIC EFFECT

565	PRIMAQUINE (P) BJ 08241	20	0	ORAL	HEC	NO
		10	0	ORAL	HEC	YES
		5	0	ORAL	HEC	NO
	QUININE (Q) BM 05870	80	0	ORAL	HEC	NO
		40	0	ORAL	HEC	NO
		20	0	ORAL	HEC	NO
	P + Q	20 + 80	0	ORAL	HEC	YES, TOXIC
		20 + 40	0	ORAL	HEC	YES, TOXIC
		20 + 20	0	ORAL	HEC	NO
		10 + 80	0	ORAL	HEC	NO
		10 + 40	0	ORAL	HEC	NO
		10 + 20	0	ORAL	HEC	NO
		5 + 80	0	ORAL	HEC	NO
		5 + 40	0	ORAL	HEC	NO
		5 + 20	0	ORAL	HEC	NO

RESULTS - ADDITIVE EFFECT

567	PRIMAQUINE (P) BJ 08241	20	0	ORAL	HEC	NO
		10	0	ORAL	HEC	NO
		5	0	ORAL	HEC	NO
	PROGUANIL (PROG)	2.5	0	ORAL	HEC	NO
		1.25	0	ORAL	HEC	NO
		0.625	0	ORAL	HEC	NO
	P + PROG	20 + 2.5	0	ORAL	HEC	YES
		20 + 1.25	0	ORAL	HEC	YES
		20 + 0.625	0	ORAL	HEC	YES
		10 + 2.5	0	ORAL	HEC	NO
		10 + 1.25	0	ORAL	HEC	NO
		10 + 0.625	0	ORAL	HEC	NO

5 + 2.5	0	ORAL	HEC	NO
5 + 1.25	0	ORAL	HEC	NO
5 + 0.625	0	ORAL	HEC	NO

RESULTS - SYNERGISTIC EFFECT

568	PRIMAQUINE (P)	20	-1, 0, +1	ORAL	HEC	YES
	BJ 08241	10	-1, 0, +1	ORAL	HEC	NO
		5	-1, 0, +1	ORAL	HEC	NO
	PROGUANIL (PROG)	0.625	-1, 0, +1	ORAL	HEC	NO
		0.3125	-1, 0, +1	ORAL	HEC	NO
		0.156	-1, 0, +1	ORAL	HEC	NO
	P + PROG	20 + 0.625	-1, 0, +1	ORAL	HEC	NO
		20 + 0.3125	-1, 0, +1	ORAL	HEC	YES
		20 + 0.156	-1, 0, +1	ORAL	HEC	NO
		10 + 0.625	-1, 0, +1	ORAL	HEC	NO
		10 + 0.3125	-1, 0, +1	ORAL	HEC	NO
		10 + 0.156	-1, 0, +1	ORAL	HEC	NO

RESULTS - ADDITIVE EFFECT

569	PRIMAQUINE (P)	20	-1, 0, +1	ORAL	HEC	YES
	BJ 08241	10	-1, 0, +1	ORAL	HEC	NO
		5	-1, 0, +1	ORAL	HEC	NO
	DAPSONE (D)	20	-1, 0, +1	ORAL	HEC	YES
		5	-1, 0, +1	ORAL	HEC	NO
		1.25	-1, 0, +1	ORAL	HEC	NO
	P + D	20 + 20	-1, 0, +1	ORAL	HEC	YES
		20 + 5	-1, 0, +1	ORAL	HEC	YES
		20 + 1.25	-1, 0, +1	ORAL	HEC	YES
		10 + 20	-1, 0, +1	ORAL	HEC	YES
		10 + 5	-1, 0, +1	ORAL	HEC	YES
		10 + 1.25	-1, 0, +1	ORAL	HEC	NO
		5 + 20	-1, 0, +1	ORAL	HEC	NO
		5 + 5	-1, 0, +1	ORAL	HEC	NO
		5 + 1.25	-1, 0, +1	ORAL	HEC	NO

RESULTS - SYNERGISTIC EFFECT

576	PRIMAQUINE (P)	20	-1, 0, +1	ORAL	HEC	NO
	BJ 08241	10	-1, 0, +1	ORAL	HEC	NO
		5	-1, 0, +1	ORAL	HEC	NO
	BQ 98852 (BQ)	40	-1, 0, +1	ORAL	HEC	YES
		20	-1, 0, +1	ORAL	HEC	YES
		10	-1, 0, +1	ORAL	HEC	YES

P + BQ	20 + 40	-1, 0, +1	ORAL	HEC	YES
	20 + 20	-1, 0, +1	ORAL	HEC	YES
	20 + 10	-1, 0, +1	ORAL	HEC	YES
	10 + 40	-1, 0, +1	ORAL	HEC	YES
	10 + 20	-1, 0, +1	ORAL	HEC	YES
	10 + 10	-1, 0, +1	ORAL	HEC	YES
	5 + 40	-1, 0, +1	ORAL	HEC	YES
	5 + 20	-1, 0, +1	ORAL	HEC	YES
	5 + 10	-1, 0, +1	ORAL	HEC	YES

RESULTS - NOT DETERMINED NEED LOWER LEVELS

578	PRIMAQUINE (P) BJ 08241	20	-1, 0, +1	ORAL	HEC	NO
		10	-1, 0, +1	ORAL	HEC	NO
		5	-1, 0, +1	ORAL	HEC	NO
	DOXYCYCLINE (D)	40	-1, 0, +1	ORAL	HEC	NO
		20	-1, 0, +1	ORAL	HEC	NO
		10	-1, 0, +1	ORAL	HEC	NO
P + D		20 + 40	-1, 0, +1	ORAL	HEC	NO
		20 + 20	-1, 0, +1	ORAL	HEC	NO
		20 + 10	-1, 0, +1	ORAL	HEC	NO
		10 + 40	-1, 0, +1	ORAL	HEC	NO
		10 + 20	-1, 0, +1	ORAL	HEC	NO
		10 + 10	-1, 0, +1	ORAL	HEC	NO
		5 + 40	-1, 0, +1	ORAL	HEC	NO
		5 + 20	-1, 0, +1	ORAL	HEC	NO
		5 + 10	-1, 0, +1	ORAL	HEC	NO

RESULTS - ADDITIVE EFFECT

581	PRIMAQUINE (P) BJ 08241	20	-1, 0, +1	ORAL	HEC	YES
		10	-1, 0, +1	ORAL	HEC	NO
		5	-1, 0, +1	ORAL	HEC	NO
	DAPSONE (D)	10	-1, 0, +1	ORAL	HEC	NO
		5	-1, 0, +1	ORAL	HEC	NO
		2.5	-1, 0, +1	ORAL	HEC	NO
P + D		20 + 10	-1, 0, +1	ORAL	HEC	YES
		20 + 5	-1, 0, +1	ORAL	HEC	YES
		20 + 2.5	-1, 0, +1	ORAL	HEC	YES
		10 + 10	-1, 0, +1	ORAL	HEC	NO
		10 + 5	-1, 0, +1	ORAL	HEC	NO
		10 + 2.5	-1, 0, +1	ORAL	HEC	NO
		5 + 10	-1, 0, +1	ORAL	HEC	NO
		5 + 5	-1, 0, +1	ORAL	HEC	NO
		5 + 2.5	-1, 0, +1	ORAL	HEC	NO

RESULTS - ADDITIVE EFFECT

Table IV Compounds Tested in the Gametocyte Test (GT)

EXP. #	COMPOUND #	MG/KG
1	Arteether	64
1	Arteether	16
1	Arteether	4
1	Arteether	1
1	Arteether	0.25
2	BH 58522	40
2	BH 58522	20
2	BH 58522	10
2	BH 58522	5
2	BH 58522	2.5
2	BH 58522	1.25
3	BJ 08241	80
3	BJ 08241	40
3	BJ 08241	20
3	BJ 08241	10
3	BJ 08241	5
3	BJ 08241	2.5
3	BJ 08241	1.25
5	Proguanil	64
5	Proguanil	32
5	Proguanil	16
5	Proguanil	8
5	Proguanil	4
6	AU 29291	64
6	AU 29291	16
6	AU 29291	4
6	AU 29291	1
6	AG 65046	4
6	AG 65046	1
6	AG 65046	0.25
7	ZB 69096	64
7	ZB 69096	16
7	ZB 69096	4
7	ZB 69096	1
7	ZB 69096	0.24
8	ATOVAQUONE	32
8	ATOVAQUONE	8
8	ATOVAQUONE	4
8	ATOVAQUONE	1
8	ATOVAQUONE	0.25
8	ATOVAQUONE	0.0625

8	ATOVAQUONE	0.03125
8	ATOVAQUONE	0.0156
9	BP 18288	64
9	BP 18288	16
9	BP 18288	4
9	BP 18288	1
9	BP 18288	0.25
9	BP 18288	0.125
9	BP 18288	0.0625
9	BP 18288	0.03125
9	BP 18288	0.0156
10	Triclosan	128
10	Triclosan	64
10	Triclosan	32
10	WR 9792 - AE07615	128
10	WR 9792 - AE07615	64
10	WR 9792 - AE07615	32
10	WR 99210 - AU20967	128
10	WR 99210 - AU20967	64
10	WR 99210 - AU20967	32
11	WR 229805, BG 75046	80
11	WR 229805, BG 75046	40
11	WR 229805, BG 75046	20
11	WR 230385, BG 81491	80
11	WR 230385, BG 81491	40
11	WR 230385, BG 81491	20
11	WR 230390, BG 81517	80
11	WR 230390, BG 81517	40
11	WR 230390, BG 81517	20
12	WR 9792, AJ 63248	80
12	WR 9792, AJ 63248	40
12	WR 9792, AJ 63248	20
12	WR 9792, AJ 63248	10
12	WR 9792, AJ 63248	5
12	WR 9792, AJ 63248	2.5
12	WR 9792, AJ 63248	1.25
12	WR 9792, AJ 63248	0.625
13	WR 238605, BK 73252	64
13	WR 238605, BK 73252	32
13	WR 238605, BK 73252	16
13	WR 238605, BK 73252	8
13	WR 238605, BK 73252	4
13	WR 238605, BK 73252	2
13	WR 238605, BK 73252	1
13	WR 238605, BK 73252	0.5

13	WR 238605, BK 73252	0.25
14	ZE 27977	128
14	ZE 27977	64
14	ZE 27977	32
14	ZE 27977	16
14	ZE 27977	8
14	ZE 27977	4
14	ZE 27977	2
14	ZE 27977	1
14	PROGUANIL	64
14	PROGUANIL	32
15	BK 11592	128
15	BK 11592	64
15	BK 11592	32
15	BK 11592	16
15	BK 11592	8
15	WR 226.377, BH 30980	64
15	WR 226.377, BH 30980	32
15	WR 226.377, BH 30980	16
15	WR 226.377, BH 30980	8
15	WR 226.377, BH 30980	4
16	AW 23860	256
16	AW 23860	128
16	AW 23860	64
16	AW 23860	32
16	BE 52678	128
16	BE 52678	64
16	BE 52678	32
16	BE 52678	16
16	BE 52678	8
17	Arteether	256
17	Arteether	128
17	Arteether	64
17	Arteether	32
17	Arteether	16
17	Arteether	8
18	BL 48816	8
18	BL 48816	4
18	BL 48816	2
18	BL 35784	8
18	BL 35784	4
18	BL 35784	2
19	Azithromycin BN 36843	128
19	Azithromycin BN 36843	64

19	Azithromycin BN 36843	32
20	BL 51822	128
20	BL 51822	64
20	BR 01096	40
20	BR 01096	20
20	BR 01096	10
20	BR 01078, WR 289048	40
20	BR 01078, WR 289048	20
20	BR 01078, WR 289048	10
20	BR 01078, WR 288568	40
20	BR 01078, WR 288568	20
20	BR 01078, WR 288568	10
21	ZE 27977	128
22	Primaquine ZE 27977	128
23	Primaquine ZE 27977	128
24	Primaquine ZE 27977	128
25	BL 48816	64
25	BL 48816	64
25	BL 48816	64
25	BJ 08241	64
25	BJ 08241	64
25	BJ 08241	64
25	AU 29291	64
25	AU 29291	64
25	AU 29291	64
26	BH 58522	64
26	BH 58522	16
26	AJ 63248	64
26	AJ 63248	16
26	BJ 08241	64
26	BJ 08241	16
26	AU 29291	64
26	BH 30980	64
27	BK 11592	64
27	BK 11592	16
27	ATOVAQUONE	64
27	ATOVAQUONE	16
27	ZB 69096	64
27	ZB 69096	16
27	AG 65046	64
27	AG 65046	16

28	WR 238605, BK 73252	64
28	BL 55866	64
28	AU 96336	64
28	BL 51822	64
29	BH 35770	64
29	BH 67852	64
29	BG 60741	64
29	BJ 91335	64
29	BJ 45646	64
29	BH 05361	64
29	BH 35761	64
30	BH 05361	64
30	BG 00478	64
30	BG 89077	64
30	BH 35761	64
30	BJ 63242	64
30	BH 10086	64
30	BH 08773	64
31	AX 66987	64
31	AX 97016	64
31	BB 43987	64
31	BC 30814	64
31	BD 22997	64
31	BH 50615	64
31	BL 56390	64
31	BM 10577	64
33	Caffeine	128
33	Caffeine	64
33	Caffeine	32
34	AY 60630	64
34	AY 62143	64
34	AY 90469	64
34	AY 63293	64
34	AY 90521	64
34	AY 64601	64
34	AY 67228	64
34	AY 67942	64
35	AF 17232	64
35	AE 38021	64
35	AE 35771	64
35	AC 73726	64
35	AC 57231	64
35	AC 56181	64
35	AD 40786	64

35	AD 40777	64
35	AD 01485	64
36	BE 19539	64
36	BE 49377	64
36	BE 49395	64
36	BE 49402	64
37	AK 19530	64
37	AS 52110	64
37	AX 61481	64
37	AX 64044	64
37	AY 91500	64
37	BG 00512	64
37	BG 80501	64
37	BG 81115	64
37	BG 94292	64
38	BL 28618	64
38	BL 47346	64
38	BL 48567	64
38	BJ 02212	64
38	BH 48053	64
38	BE 43914	64
38	BE 16387	64
39	Mangostin	64
39	7-ethyl-10,11-MD-20(s)-CPT	64
39	7-ethyl-10,11-MP-20(s)-CPT Glester-HCl	64
39	Nitidine	64
39	6-methoxy-5,6-dihydro-nitidine	64
39	AS 67317	64
39	AS 32850	64
39	BM 10371	64
39	ZN 39888	64
39	AH 91158	64